



## Effect of soluble solids concentration on *Neosartorya fischeri* inactivation using UV-C light



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### ABSTRACT

Ascospores of *Neosartorya fischeri* are heat-resistant and can survive thermal commercial treatments normally applied to the juices, as apple juice. Non-thermal processing of food such as exposure to ultraviolet light (UV-C) is reported to induce minimal quality changes while reduces microbial load. The main objective of this study was to determine the effect at different soluble solids concentration (12, 25, 30, 40, 50, 60 and 70 °Brix) on *N. fischeri* ascospores inactivation in apple juice, using UV-C light intensity (38 W/m<sup>2</sup>). Weibull model was fitted to experimental data. Then, a secondary model was used to describe how the inactivation kinetic parameters varied with the changes in soluble solids concentration. Results showed that the UV-C light had influence on *N. fischeri* ascospores inactivation in apple juice even at the highest soluble solids concentrations used, reaching approximately 4 log reductions at all concentrations used. The inactivation parameters, obtained by Weibull model, were  $\delta$  (dose for the first decimal reduction) and  $p$  (the shape factor). Exponential model was chosen to describe the influence of soluble solids concentration on  $\delta$  and  $p$  parameters. It can be concluded that UV-C light is a promising treatment with a drastic impact on the loads of *N. fischeri*, especially when low soluble solids concentration is used and a model was obtained to describe Brix effect.

### 1. Introduction

Moulds generally associated with spoilage of pasteurized fruit juices, as apple juice and other fruit products are often heat-resistant. Ascospores of these moulds are extremely heat tolerant compared with those of other fungi. Among the heat-resistant moulds known to survive pasteurization treatments sometimes given to fruit juices is *Neosartorya fischeri* (Beuchati and Kuhn, 1997).

*N. fischeri* moulds produce extremely heat resistant spores inside an ascus mother cell, called ascospores. They are able to grow over a wide range of temperatures (10 to 52 °C), pH (3 to 8) and at low oxygen partial pressures (Evelyn and Silva, 2017; Nielsen et al., 1988). Pitt and Hocking (2009) reported that the degree of heat resistance of *N. fischeri* ascospores is close to that of many bacterial spores and is higher than *Byssoschlamys fulva* ascospores. The capability to produce mycotoxins terrein, fumitremorgins A and B, and verruculogen makes *N. fischeri* causes public health concern (Nielsen et al., 1988; Tournas and Traxler, 1994).

Pasteurization temperatures are commonly used to prolong the shelf life of fruit juices (Sant'Ana et al., 2009). However, it has been

recognized that the thermal process can stimulate the dormant ascospores of moulds responsible for economic losses and food spoilage (Berni et al., 2017; Slongo and De Aragão, 2006). dos Santos et al. (2018) identified heat resistant moulds ascospores from samples collected on the pasteurized treatment of high acidity fruit products, where 26.7% of the orange juice pasteurized samples were predominantly contaminated by *N. fischeri*. Increasing the intensity (temperature or processing time) of the heat treatment is not desirable, due to quality reasons and consumer demands for 'fresh-like' fruit products. Non-thermal methods have been investigated due to reduced treatment temperatures and processing times, such as ultrasound, pulsed electric field, high pressure processing (HPP) and ultraviolet light (Estilo and Gabriel, 2018; Evelyn and Silva, 2018; Li et al., 2017; Mikš-Krajnc et al., 2017; Tremarin et al., 2017; Uchida and Silva, 2017).

Ultraviolet (UV) light is the part of the electromagnetic spectrum with wavelengths between 100 and 400 nm. The highest germicidal effect is between 250 and 280 nm (UV-C light). Possible use of UV light in liquid foods has been tested on orange juice (Tran and Farid, 2004), apple cider (Koutchma et al., 2004), milk (Krishnamurthy et al., 2007) and liquid egg derivate (Unluturk et al., 2008), with growing

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applications. UV light may also improve food safety by reducing patulin, a mycotoxin present in fresh apple cider (Dong et al., 2010). UV-light treatment of liquid foods can be performed by use of liquid foods can be applied as a continuous flow. UV-C (wavelengths of 220–300 nm with 90% emission at 253.7 nm) has been approved by the FDA as a mean for controlling surficial microorganisms (biofilm) on food products (US Food and Drug Administration, 2004). In the fruit juices industry, soluble solids (expressed in °Brix) is one major factor influencing on inactivation kinetics of heat-resistant fungi (Estilo and Gabriel, 2018; Souza et al., 2017). It is known that the germicidal efficacy of UV-C processing depends on the soluble solids (sugars, organic acids, etc.), insoluble suspended matter (insoluble fibers), flow characteristics (laminar or turbulent) (Guerrero-Beltrán and Barbosa-Cánovas, 2005; Koutchma et al., 2009). However, until our knowledge, there is no literature report on modeling the effect of soluble solids on the inactivation of *N. fischeri* spores using UV-C lights in concentrate fruit juice. The aims of this study were to determine and model the effect of soluble solids concentration on the inactivation of *N. fischeri* ascospores in apple juice using different doses of UV-C light.

## 2. Material and methods

### 2.1. Spores suspension

The mould *Neosartorya fischeri* was isolated from an apple nectar processing plant and identified by Salomão et al., 2007. *N. fischeri* ascospores were obtained after pre-sporulation in Petri dishes containing Potato Dextrose Agar (PDA) medium (pH 3.5) at 30 °C for seven days. Collected spores were added to the plates containing Malt Extract Agar (MEA, Kasvi®, Brazil) medium and incubated for 30 days at 30 °C. The spores were collected by flooding the surface of the culture plates with 10 mL sterile distilled water and scraped from the agar surface with a sterile rubber spatula. The spore suspension was subsequently filtered through four layers of sterile gauze and centrifuged at 3500 rpm (2000 times the force of gravity) for 10 min at 5 °C and the procedure was repeated five times and until no hyphae were seen under the microscope. The final suspension was prepared with the precipitate in a minimum volume of water, sufficient to obtain a highly concentrated suspension. The supernatant was then removed, re-suspended in sterile water and kept in refrigerated conditions until further use (Salomão et al., 2007). Concentration of spores' suspension was around 10<sup>6</sup> CFU/mL.

### 2.2. Fruit juice samples

Concentrate commercial apple juice (pH 3.6, 70.5 ± 0.1 °Brix) was obtained from Fischer S.A. and used as the medium to suspend and process the ascospores. In the adjustment of soluble solids (SS) to the desired °Brix values, appropriate amounts of distilled water were dissolved in apple juice to obtain the concentrations of 12, 25, 30, 40, 50, 60 and 70 °Brix. The soluble solid content (°Brix) was determined by a refractometer (AR200 Reichert, USA). Each juice sample had a volume of 25 mL of apple juice artificially inoculated with 0.1 mL of ascospores suspension (initial spore concentration was approximately 10<sup>6</sup> CFU/mL of juice).

### 2.3. Ultraviolet inactivation treatments

Juice exposure to UV-C light was done inside a chamber (40 × 40 × 40 cm) with ten germicidal lamps (DULUX HNS L24W, OSRAM, Germany) with peak emission at 254 nm. Juice samples were placed in Petri dishes 25 cm below the lamps and continuously stirred during the irradiation with a magnetic agitation. The juice samples had a deepness of 4 mm. The intensity of the lamp was measured by a UV-VIS radiometer (DELTA OHM HD 2302.0, Italy) at the same distance from the UV lamp as the plates, giving a corresponding intensity

of 38 W/m<sup>2</sup>. UV doses were calculated by multiplying the UV intensities (38 W/m<sup>2</sup>) by the exposure times (0, 1, 2, 4, 7, 10, 15, 20, 30, 35, 40 min). At a given SS concentration, samples of 1 mL were taken after 0, 1, 2, 4, 7, 10, 15, 20, 30, 35, 40 min of exposure. The UV lamp was switched on for about 30 min prior to UV-C treatment to minimize intensity fluctuations.

After each treatment, microbiological analyses of the juices were carried out. At least three replicates of each treatment were performed.

### 2.4. Microbiological analysis

After inactivation treatments, survival spores in juice samples were determined by pour plating. Spore samples were decimal diluted using 9 mL 0.1% (w/v) sterile buffered peptone water. Then, 1 mL of each dilution was transferred to sterile Petri dishes with agar PDA (Potato, Dextrose, Agar) broth (Kasvi®, Brazil). The plates were incubated at 30 °C for 3–5 days. Plates with 20 to 100 colonies were used for enumeration. Microbial counts were performed in triplicate and expressed as CFU/mL.

### 2.5. Modeling the ascospore inactivation and data analysis

MATLAB R2013a (MathWorks®, Natick, USA) was used to fit a Weibull model to UV-C spore survival lines, and to estimate the model parameters for *N. fischeri* ascospores in apple juice.

The Weibull distribution (Eq. (1)) written in the decimal logarithmic form is as follows (Mafart et al., 2001):

$$\log N = \log N_0 - (d/\delta)^p \quad (1)$$

where:  $N$  (population at time  $t$ ),  $N_0$  (initial population),  $d$  (dose (kJ/m<sup>2</sup>)),  $\delta$  (the scale factor) and  $p$  (shape parameter).  $\delta$  (kJ/m<sup>2</sup>) is the UV dose for the first decimal reduction. The term  $p$  (dimensionless) is the survival curve shape factor:  $p < 1$  and  $p > 1$  correspond to survival curves with concave upwards (tailings) and concave downwards (shoulders), respectively. When  $p = 1$ ; the Weibull model becomes a simple first-order kinetics.

For each SS concentration, three survival experiments were performed for UV-C treatments. For each survival experiment, duplicate samples were processed for each time. Then, the  $\log(N)$  were expressed as mean ± standard deviation (SD), which includes the result of seven different concentration samples and the average data were plotted in a chart. For each SS, the model parameters ± standard errors (SE) were estimated by fitting the models to the average of the data points for each processing time. Root mean square error (RMSE) and coefficient of determination ( $R^2$ ) were used to obtain the performance of the model. A relatively small MSE and  $R^2$  values close to 1 indicate the adequacy of the model to describe the survival data.

Secondary models normally used for modeling growth and inactivation parameters of bacteria have been used for parameters of moulds (Dantigny et al., 2005). After obtaining the primary parameters of the scale factor ( $\delta$ ) and the form factor ( $p$ ), the Square Root (Ratkowsky et al., 1982) and the exponential secondary models (Eqs. (2) and (3), respectively) were used to describe the influence of soluble solids concentration (Baranyi et al., 1995; Haberbeck et al., 2012; Longhi et al., 2013; Menezes et al., 2018). The fitting procedure was performed in Matlab R2013a (The MathWorks Inc., Natick, USA).

$$\sqrt{y} = b(SS - SS_{\min}) \quad (2)$$

$$y = a \cdot \exp(cSS) \quad (3)$$

where  $a$ ,  $b$  and  $c$  are empirical parameters of the equation;  $SS$  corresponds to the soluble solids concentration values and  $y$  corresponds to the parameters  $\delta$  or  $p$ .

Statistical indices were used for validation the inactivation models: coefficient of determination ( $R^2$ ), Eq. (4) and root mean squared error (RMSE), Eq. (5) (Ross, 1996). A relatively small RMSE and  $R^2$  values

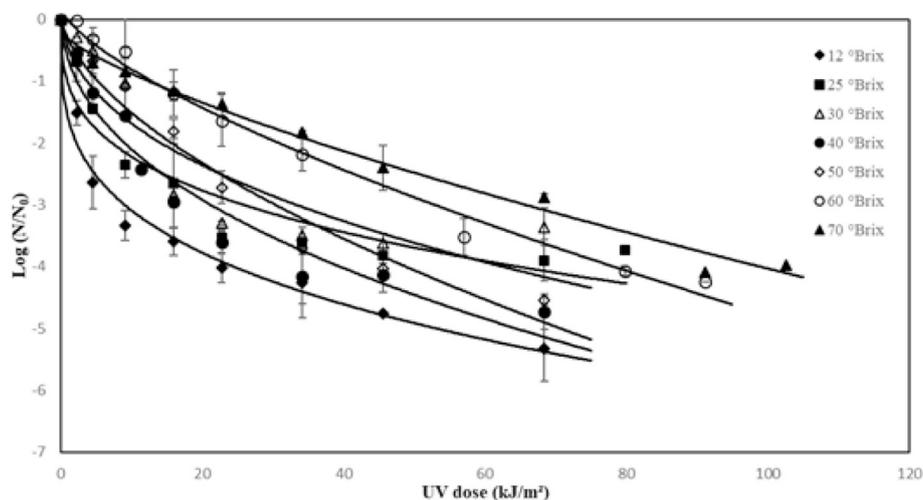


Fig. 1. *Neosartorya fischeri* ascospores inactivation in apple juice applying different soluble solids concentration at UV intensity of 38 kJ/m<sup>2</sup>. Continues lines represent Weibull model fits for each condition individually. Error bars indicate about the triplicates of the experiment.

close to 1 indicate the adequacy of the model to describe the survival data.

$$R^2 = 1 - \frac{\sum_{i=1}^n (\text{pred} - \text{obs})^2}{\sum_{i=1}^n (\text{obs} - \text{mean})^2} \quad (4)$$

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^n (\text{pred} - \text{obs})^2}{n - p}} \quad (5)$$

### 3. Results and discussion

#### 3.1. Ascospore inactivation of *N. fischeri* by UV-C light

Experimental inactivation data of *N. fischeri* ascospores in apple juice by UV-C light, using different soluble solids concentration (12, 25, 30, 40, 50, 60 and 70 °Brix), are shown in Fig. 1. All data are expressed in terms of log (N/N<sub>0</sub>) to avoid the influence of the initial microbial loads differences. The UV-C light affected *N. fischeri* inactivation in apple juice even at the highest soluble solids concentrations used, reaching approximately 4 log reductions at all applied concentrations.

The UV-C treatment was able to inactivate 5 log of *N. fischeri* ascospores in apple juice after 20 min at the UV intensity of 38 W/m<sup>2</sup>. Evelyn et al. (2016), evaluated the inactivation of *N. fischeri* in apple juice (pH 3.7, 10.6 ± 0.1 °Brix) using thermal treatment at 85 and 90 °C. To obtain 5 log reductions, 70 min at 85 °C or 20 min at 90 °C were required. The same authors also used non-thermal methods as high pressure processing (HPP) and power ultrasound in combination with mild heat, HPP-thermal and therosonication (TS) for *N. fischeri* inactivation and found approximately 5 log reductions using HPP 600 MPa and 3.5 log reductions using TS (24 RHz, 0.33 W/mL) in 40 min at 75 °C. Sant'Ana et al. (2009) worked with thermal treatment of *B. fulva* ascospores in clarified apple juice (11 °Brix), to obtain 5 log reduction at 85 °C around 90 min were needed and 15 to 20 min at 90 °C.

García Carrillo et al. (2017) examined the effect of short wave ultraviolet light (UV-C, 0 to 10.6 kJ/m<sup>2</sup>) assisted by mild heat treatment (40, 45 or 50 °C) on *Escherichia coli*, *Saccharomyces cerevisiae* and *Pseudomonas fluorescens* inactivation in freshly squeezed carrot-orange juice blend (pH 3.8, 10.6 °Brix). They find (UV-C - 10.6 kJ/m<sup>2</sup> and heat treatment 50 °C) 4.2 log reductions for *S. cerevisiae* and > 6.0 log reductions for *P. fluorescens* and *E. coli*. Results showed that UV-C light is least effective against fungi and spores than against bacteria (Koutchma et al., 2009).

In the present work, UV-C treatment, even at high soluble solids concentration, was more efficient in microbial reduction than other methods as high pressure thermal processing (HPTP), therosonication (TS) and thermal treatment at 85 and 90 °C, studied for other authors. Accordingly, experimental results obtained for the inactivation of *N. fischeri* in apple juice allowed concluding that UV-C treatment at the intensity tested is a promising application for preventing the spoilage of juices.

The influence of soluble solids concentration in *N. fischeri* spore's inactivation using UV-C light was observed on Fig. 1. In the lowest soluble solid concentration (12 °Brix), the required UV-dose to achieve an inactivation of 5 log units was found as approximately 53 kJ/m<sup>2</sup>, which corresponds to an intensity of intensity of 38 W/m<sup>2</sup> for 20 min. The suggested UV-doses for approximately 4.2 log reductions at the highest concentrations (60 and 70 °Brix) was > 80 kJ/m<sup>2</sup> or 35 min at the UV intensity of 38 W/m<sup>2</sup>, showing a protective effect of soluble solids concentration in the *N. fischeri* inactivation using UV-C light. The knowledge of the soluble solids is highly important factor affecting microbial inactivation using UV-C light in fruit juices. According to Koutchma (2009), the increasing amount of dissolved organic solids in a liquid system results in significant attenuation in the antimicrobial efficacies of UV-C.

Several studies have previously described the significant effects of soluble solids concentration on microorganisms inactivation (Estilo and Gabriel, 2018; Souza et al., 2017; Uchida and Silva, 2017). Estilo and Gabriel (2018) studied the influence of soluble and insoluble solids, and treated volume on the UV-C resistance of *Salmonella enterica*. The authors found, at the same insoluble solids (1.5%) and treated volume (625 mL) a large variation in the decimal reduction time (D<sub>UV-C</sub>-value), calculated from the inactivation curves. The D<sub>UV-C</sub> results obtained

Table 1

Weibull model parameters for the survival of *Neosartorya fischeri* ascospores of different soluble solids after UV-C processing.

Soluble solids (°Brix)	$\delta \pm \text{se}$ (kJ/m <sup>2</sup> )	$p \pm \text{se}$	R <sup>2</sup>	RMSE
12	0.18 ± 0.34	0.28 ± 0.09	0.98	0.284
25	0.54 ± 1.77	0.30 ± 0.17	0.91	0.463
30	1.87 ± 7.11	0.42 ± 0.39	0.84	0.713
40	1.41 ± 2.70	0.43 ± 0.19	0.95	0.428
50	4.38 ± 5.35	0.59 ± 0.24	0.97	0.334
60	9.89 ± 6.06	0.69 ± 0.18	0.99	0.202
70	16.29 ± 8.31	0.74 ± 0.18	0.99	0.180

**Table 2**

Estimated parameter values (a, b, c and  $SS_{\min}$ ) of the fitting of Square Root and Exponential secondary models to  $\delta$  and  $p$  parameters values and the statistical indexes ( $R^2$  and RMSE).

Parameter	Square root				Exponential			
	b (kJ <sup>0.5</sup> /m <sup>0.5</sup> B)	$SS_{\min}$ (°B)	$R^2$	RMSE	a (kJ/m <sup>2</sup> )	c	$R^2$	RMSE
$\delta$	0.062	11.5	0.92	0.751	0.093	0.076	0.95	0.604
$p$	0.006	-68.9	0.95	0.039	0.219	0.018	0.95	0.043

were 10, 29 and 60.8 (mJ/cm<sup>2</sup>) for 0, 35 and 70 °Brix, respectively in simulated fruit juices.

Koutchma (2009) reported that soluble solids, light absorbing ingredients, and suspended particles in food products substantially decrease the penetration depth and availability of the UV-C light photons, which consequently affects microbial inactivation. Transparency is often influenced by the chemical composition of fluids as well as the concentration of dissolved and suspended solids.

### 3.2. Modeling the UV-C inactivation of *N. fischeri* ascospores in apple juice

The Weibull model was able to describe *N. fischeri* inactivation and showed the best fits, confirming the performance through statistical indexes and estimated parameters ( $\delta$  and  $p$ ), presented in Table 1. Analyzing the results of Weibull model,  $p$  values (shape factors) were between 0.28 and 0.74 ( $p < 1$ ), indicating an upward concavity. The concave upwards shape of the curves corresponds to a tailing phase in which the inactivation rate decreases, this may be related to the solids in suspension that block the UV-light through the system, non-homogeneous treatments, aggregation of microorganisms or the presence of resistant subpopulation (Baysal et al., 2013; Hijnen et al., 2006). The use of combined mild heat and UV-C treatments could be one solution to decrease the spore resistance observed with processing time. The scale parameter ( $\delta$ ) represents the time for the first decimal reduction and found as increased from 0.08 at 12 °Brix to 7.14 min at 70 °Brix, demonstrating this parameter is dependent within this range of soluble solids concentration. These results were in agreement with several studies reported with other microorganisms where the Weibull model could quantitatively describe microbial inactivation by UV-C in different food matrices (Baysal et al., 2013; García Carrillo et al., 2017; Hakguder Taze et al., 2013; Unluturk and Atilgan, 2014).

The results obtained in this work corroborate previous studies that have also reported  $p$  values  $< 1$ . Flores-Cervantes et al. (2013) characterized survival curves of *Aspergillus flavus* and *Aspergillus niger* spores in peach nectar processed by UV-C light alone (203 kJ/m<sup>2</sup>) or combined with antimicrobials by Weibull model, reporting  $p$  values  $< 1$  (0.19 to 0.72) for the inactivation of both microorganisms. García Carrillo et al.

(2017) fitted Weibull model to survival curves of *Escherichia coli*, *S. cerevisiae* and *Pseudomonas fluorescens* in freshly squeezed carrot-orange juice blend exposed to UV-C light (0–10.6 kJ/m<sup>2</sup>) assisted by mild heat treatment (UV-C/H, 40, 45 or 50 °C), they also reported  $p$  values of less one.

According to the results obtained in the present study, Weibull model resulted in good performance statistical indices (0.181–0.714 RMSE and 0.84–0.99  $R^2$ ). These  $R^2$  and RMSE values are at the score present at the literature for other heat resistant moulds and spore-forming bacterium. Baysal et al. (2013) fitted Weibull model to the survival curve of *Alicyclobacillus acidoterrestris* and they found RMSE values 0.213, 0.295, 0.422 at the intensities 0.38, 0.71 and 1.31 mW/cm<sup>2</sup>, respectively for inactivation data in grape juice.

The estimated parameter values ( $\pm$  95% confidence interval) (a, b, c and  $SS_{\min}$ ) of the fitting of Square Root (Eq. (2)) and Exponential secondary models (Eq. (3)) to  $\delta$  and  $p$  parameters values and the statistical indexes ( $R^2$  and RMSE) are shown in Table 2. Both of models could describe the influence of soluble solids concentration on  $\delta$  and  $p$  parameters of Weibull due to its good performance (high  $R^2$  and low RMSE values). The square root model was chosen because is often used in the literature for modeling the effect of temperature or others factor on the parameters of the primary models. The Square Root model (Fig. 2 (a) and (b)) can be used to predict the *N. fischeri* inactivation in other soluble solids values in the studied range. To attend the growing demand from consumers for health food products and the fact that industry aims diversification of their products (such as fruit products with different and high soluble solids contents), it's important the knowledge about the factors affecting microbial inactivation, such as the relation between UV-C light intensity and the soluble solids content.

## 4. Conclusions

The primary model that best described *N. fischeri* inactivation by UV-C treatment over time was the Weibull model, where the shape parameter ( $p$ ) and the scale parameter ( $\delta$ ) were calculated for the different soluble solids concentration. To describe the variation of the shape and scale parameters with the variation of the soluble solids

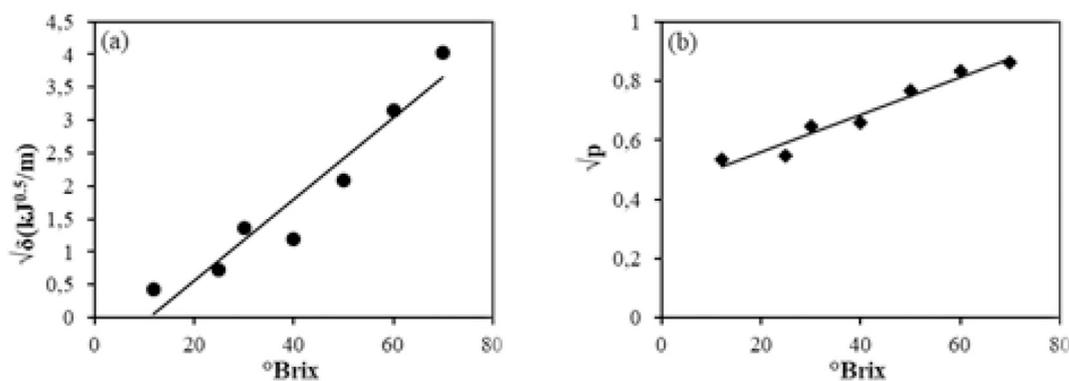


Fig. 2. Fitting Square Root secondary models (continuous lines) to describe the influence of the soluble solids concentration on the primary parameters  $\delta$  (●) and  $p$  (◆).

concentration, the secondary model fit the data. Both have good performance indices, proving that primary and secondary models have high predictive capacity.

Inactivation kinetics showed that the lowest soluble solids concentration needs less inactivation time than the other concentrations to achieve more than five logarithmic reductions were achieved.

Ultraviolet inactivation technology could be used as an alternative to thermal treatment for fruit juice products with the great advantage that it can reduce of sensory and nutritional impacts. Long treatment time is still needed to achieve a 5 log inactivation under de UV-C process. Thus, future studies should focus on the optimization of processing parameters to inactivate the *N. fischeri* spores or the combination with other methods (hurdles), such as use of mild heat and UV-C treatments. This research opens new opportunities of investigation that embrace studies on quality characteristics of the products and safety standards.

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